SEQUENCE LISTING

<110> CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE <120> GLUTAMINE: FRUCTOSE-6-PHOSPHATE AMIDOTRANSFERASE (GFAT) COMPRISING AN INTERNAL PURIFICATION TAG, AND ITS USE FOR THE SCREENING OF COMPOUNDS <130> WOB 03 BP CNR GFAT <160> 19 <170> PatentIn version 3.1 <210> <211> 2046 <212> DNA <213> Homo sapiens <220> <221> CDS <222> (1)..(2046)<223> <220> <221> misc_feature <222> (170)..(170)<223> t or c <400> 1 atg tgt ggt ata ttt gct tac tta aac tac cat gtt cct cga acg aga 48 Met Cys Gly Ile Phe Ala Tyr Leu Asn Tyr His Val Pro Arg Thr Arg cga gaa atc ctg gag acc cta atc aaa ggc ctt cag aga ctg gag tac 96 Arg Glu Ile Leu Glu Thr Leu Ile Lys Gly Leu Gln Arg Leu Glu Tyr 20 25 aga gga tat gat tct gct ggt gtg gga ttt gat gga ggc aat gat aaa 144 Arg Gly Tyr Asp Ser Ala Gly Val Gly Phe Asp Gly Gly Asn Asp Lys 35 gat tgg gaa gcc aat gcc tgc aaa anc cag ctt att aag aag aaa qqa 192 Asp Trp Glu Ala Asn Ala Cys Lys Xaa Gln Leu Ile Lys Lys Gly 50 aaa gtt aag gca ctg gat gaa gaa gtt cac aag caa caa gat atg gat 240 Lys Val Lys Ala Leu Asp Glu Glu Val His Lys Gln Gln Asp Met Asp 65 70 ttg gat ata gaa ttt gat gta cac ctt gga ata gct cat acc cgt tgg 288 Leu Asp Ile Glu Phe Asp Val His Leu Gly Ile Ala His Thr Arg Trp 85 gca aca cat gga gaa ccc agt cct gtc aat agc cac ccc cag cgc tct 336 Ala Thr His Gly Glu Pro Ser Pro Val Asn Ser His Pro Gln Arg Ser

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Ile Met Arg Asp His Thr Tyr Ala Lys Cys Gln Asn Ala Leu Gln Gln 595 600 605	1824
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act gag acc att aag aac aca aaa aga acg atc aag gtg ccc cac tca Thr Glu Thr Ile Lys Asn Thr Lys Arg Thr Ile Lys Val Pro His Ser 625 630 635 640	1920
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Arg Gin Val Leu Giu Giu Leu Thr Glu Leu Pro Val Met Val Glu Leu 385 390 395 400

Ala Ser Asp Phe Leu Asp Arg Asn Thr Pro Val Phe Arg Asp Asp Val 405 410 415

Cys Phe Phe Leu Ser Gln Ser Gly Glu Thr Ala Asp Thr Leu Met Gly 420 425 430

Leu Arg Tyr Cys Lys Glu Arg Gly Ala Leu Thr Val Gly Ile Thr Asn 435 440 445

Thr Val Gly Ser Ser Ile Ser Arg Glu Thr Asp Cys Gly Val His Ile

Asn Ala Gly Pro Glu Ile Gly Val Ala Ser Thr Lys Ala Tyr Thr Ser 475 Gln Phe Val Ser Leu Val Met Phe Ala Leu Met Met Cys Asp Asp Arg 485 490 Ile Ser Met Gln Glu Arg Arg Lys Glu Ile Met Leu Gly Leu Lys Arg 505 Leu Pro Asp Leu Ile Lys Glu Val Leu Ser Met Asp Asp Glu Ile Gln 520 525 Lys Leu Ala Thr Glu Leu Tyr His Gln Lys Ser Val Leu Ile Met Gly 535 Arg Gly Tyr His Tyr Ala Thr Cys Leu Glu Gly Ala Leu Lys Ile Lys 550 Glu Ile Thr Tyr Met His Ser Glu Gly Ile Leu Ala Gly Glu Leu Lys 570 His Gly Pro Leu Ala Leu Val Asp Lys Leu Met Pro Val Ile Met Ile 585 Ile Met Arg Asp His Thr Tyr Ala Lys Cys Gln Asn Ala Leu Gln Gln 600 Val Val Ala Arg Gln Gly Arg Pro Val Val Ile Cys Asp Lys Glu Asp 610 615 Thr Glu Thr Ile Lys Asn Thr Lys Arg Thr Ile Lys Val Pro His Ser 625 630 635 640 Val Asp Cys Leu Gln Gly Ile Leu Ser Val Ile Pro Leu Gln Leu Leu 645 650 Ala Phe His Leu Ala Val Leu Arg Gly Tyr Asp Val Asp Phe Pro Arg Asn Leu Ala Lys Ser Val Thr Val Glu 675 <210> 3 <211> 2049 <212> DNA <213> Homo sapiens <220> <221> CDS <222> (1)..(2049)<223> <400> 3 atg tgc gga atc ttt gcc tac atg aac tac aga gtc ccc cgg acg agg Met Cys Gly Ile Phe Ala Tyr Met Asn Tyr Arg Val Pro Arg Thr Arg

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						cac His										384	
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						aag Lys										480	
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					cgg Arg											912
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		_			atg Met	_	-				_	_		_		1008
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						tct Ser					tga		٠		٠		2049
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·		O> Cys		Ile	Phe 5	Ala	Tyr	Met	Asn	Tyr 10	Arg	Val	Pro	Arg	Thr 15	Arg	

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Arg Gly Tyr Asp Ser Ala Gly Val Ala Ile Asp Gly Asn Asn His Glu

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Val Lys Glu Arg His Ile Gln Leu Val Lys Lys Arg Gly Lys Val Lys 50 55 60

Ala Leu Asp Glu Glu Leu Tyr Lys Gln Asp Ser Met Asp Leu Lys Val 65 70 75 80

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Asn Glu Phe Val Val Ile His Asn Gly Ile Ile Thr Asn Tyr Lys Asp 115 120 125

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Glu Thr Glu Asp Ile Thr Phe Ser Thr Leu Val Glu Arg Val Ile Gln 165 170 175

Gln Leu Glu Gly Ala Phe Ala Leu Val Phe Lys Ser Val His Tyr Pro 180 185 190

Gly Glu Ala Val Ala Thr Arg Arg Gly Ser Pro Leu Leu Ile Gly Val 195 200 205

Arg Ser Lys Tyr Lys Leu Ser Thr Glu Gln Ile Pro Ile Leu Tyr Arg 210 215 . 220

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Lys Leu Ser Ile His Arg Val Lys Arg Ser Ala Ser Asp Asp Pro Ser 290 295 300

Arg Ala Ile Gln Thr Leu Gln Met Glu Leu Gln Gln Ile Met Lys Gly 30.5 310 315 320

Asn Phe Ser Ala Phe Met Gln Lys Glu Ile Phe Glu Gln Pro Glu Ser 325 330 335

Val Phe Asn Thr Met Arg Gly Arg Val Asn Phe Glu Thr Asn Thr Val

340 345 350

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						aag Lys 375										1152
cgg Arg	cgt Arg	ttg Leu	att Ile	ctt Leu	att Ile	gct Ala	tgt Cys	gga Gly	aca Thr	agt Ser	tac Tyr	cat His	gct Ala	ggt Gly	gta Val	1200

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aaa cgg ctg cct gat ttg att aag gaa gta ctg agc atg gat gac gaa Lys Arg Leu Pro Asp Leu Ile Lys Glu Val Leu Ser Met Asp Asp Glu att cag aaa cta gca aca gaa ctt tat cat cag aag tca gtt ctg ata Ile Gln Lys Leu Ala Thr Glu Leu Tyr His Gln Lys Ser Val Leu Ile atg gga cga ggc tat cat tat gct act tgt ctt gaa ggg gca ctg aaa Met Gly Arg Gly Tyr His Tyr Ala Thr Cys Leu Glu Gly Ala Leu Lys atc aaa gaa att act tat atg cac tct gaa ggc atc ctt gct ggt gaa Ile Lys Glu Ile Thr Tyr Met His Ser Glu Gly Ile Leu Ala Gly Glu ttg aaa cat ggc cct ctg gct ttg gtg gat aaa ttg atg cct gtg atc Leu Lys His Gly Pro Leu Ala Leu Val Asp Lys Leu Met Pro Val Ile atg atc atc atg aga gat cac act tat gcc aag tgt cag aat gct ctt Met Ile Ile Met Arg Asp His Thr Tyr Ala Lys Cys Gln Asn Ala Leu cag caa gtg gtt gct cgg cag ggg cgg cct gtg gta att tgt gat aag Gln Gln Val Val Ala Arq Gln Gly Arq Pro Val Val Ile Cys Asp Lys

630

150

635

640

625 gag gat act gag acc att aag aac aca aaa aga acg atc aag gtg ccc 1968 Glu Asp Thr Glu Thr Ile Lys Asn Thr Lys Arg Thr Ile Lys Val Pro 650 645 cac tca gtg gac tgc ttg cag ggc att ctc agc gtg atc cct tta cag 2016 His Ser Val Asp Cys Leu Gln Gly Ile Leu Ser Val Ile Pro Leu Gln 665 2064 ttg ctg gct ttc cac ctt gct gtg ctg aga ggc tat gat gtt gat ttc Leu Leu Ala Phe His Leu Ala Val Leu Arg Gly Tyr Asp Val Asp Phe 680 675 2100 cca cgg aat ctt gcc aaa tct gtg act gta gag tga Pro Arg Asn Leu Ala Lys Ser Val Thr Val Glu <210> 6 <211> 699 <212> PRT <213> Homo sapiens <220> <221> misc_feature (57)..(57)'Xaa' in position 57 represents Thr or Ile Met Cys Gly Ile Phe Ala Tyr Leu Asn Tyr His Val Pro Arg Thr Arg Arg Glu Ile Leu Glu Thr Leu Ile Lys Gly Leu Gln Arg Leu Glu Tyr Arg Gly Tyr Asp Ser Ala Gly Val Gly Phe Asp Gly Gly Asn Asp Lys Asp Trp Glu Ala Asn Ala Cys Lys Xaa Gln Leu Ile Lys Lys Gly Lys Val Lys Ala Leu Asp Glu Glu Val His Lys Gln Gln Asp Met Asp Leu Asp Ile Glu Phe Asp Val His Leu Gly Ile Ala His Thr Arg Trp Ala Thr His Gly Glu Pro Ser Pro Val Asn Ser His Pro Gln Arg Ser 105 Asp Lys Asn Asn Glu Phe Ile Val Ile His Asn Gly Ile Ile Thr Asn 120 115 Tyr Lys Asp Leu Lys Lys Phe Leu Glu Ser Lys Gly Tyr Asp Phe Glu 135 Ser Glu Thr Asp Thr Glu Thr Ile Ala Lys Leu Val Lys Tyr Met Tyr

Asp Asn Arg Glu Ser Gln Asp Thr Ser Phe Thr Thr Leu Val Glu Arg 165 Val Ile Gln Gln Leu Glu Gly Ala Phe Ala Leu Val Phe Lys Ser Val 185 His Phe Pro Gly Gln Ala Val Gly Thr Arg Arg Gly Ser Pro Leu Leu 200 Ile Gly Val Arg Ser Glu His Lys Leu Ser Thr Asp His Ile Pro Ile 215 Leu Tyr Arg Thr Ala Arg Thr Gln Ile Gly Ser Lys Phe Thr Arg Trp 230 235 Gly Ser Gln Gly Glu Arg Gly Lys Asp Lys Gly Ser Cys Asn Leu Ser Arg Val Asp Ser Thr Thr Cys Leu Phe Pro Val Glu Glu Lys Ala Val Glu Tyr Tyr Phe Ala Ser Asp Ala Ser Ala Val Ile Glu His Thr 280 Asn Arg Val Ile Phe Leu Glu Asp Asp Asp Val Ala Ala Val Val Asp 295 Gly Arg Leu Ser Ile His Arg Ile Lys Arg Thr Ala Gly Asp His Pro 315 310 Gly Arg Ala Val Gln Thr Leu Gln Met Glu Leu Gln Gln Ile Met Lys Gly Asn Phe Ser Ser Phe Met Gln Lys Glu Ile Phe Glu Gln Pro Glu 345 Ser Val Val Asn Thr Met Arg Gly Arg Val Asn Phe Asp Asp Tyr Thr 355 Val Asn Leu Gly Gly Leu Lys Asp His Ile Lys Glu Ile Gln Arg Cys Arg Arg Leu Ile Leu Ile Ala Cys Gly Thr Ser Tyr His Ala Gly Val 385 Ala Thr Arg Gln Val Leu Glu Glu Leu Thr Glu Leu Pro Val Met Val 405 Glu Leu Ala Ser Asp Phe Leu Asp Arg Asn Thr Pro Val Phe Arg Asp 425 Asp Val Cys Phe Phe Leu Ser Gln Ser Gly Glu Thr Ala Asp Thr Leu Met Gly Leu Arg Tyr Cys Lys Glu Arg Gly Ala Leu Thr Val Gly Ile 450 455 Thr Asn Thr Val Gly Ser Ser Ile Ser Arg Glu Thr Asp Cys Gly Val His Ile Asn Ala Gly Pro Glu Ile Gly Val Ala Ser Thr Lys Ala Tyr 485 490 495

Thr Ser Gln Phe Val Ser Leu Val Met Phe Ala Leu Met Met Cys Asp 500 505 510

Asp Arg Ile Ser Met Gln Glu Arg Arg Lys Glu Ile Met Leu Gly Leu 515 520 525

Lys Arg Leu Pro Asp Leu Ile Lys Glu Val Leu Ser Met Asp Asp Glu 530 535 540

Ile Gln Lys Leu Ala Thr Glu Leu Tyr His Gln Lys Ser Val Leu Ile 545 550 560

Met Gly Arg Gly Tyr His Tyr Ala Thr Cys Leu Glu Gly Ala Leu Lys
565 570 575

Ile Lys Glu Ile Thr Tyr Met His Ser Glu Gly Ile Leu Ala Gly Glu 580 585 590

Leu Lys His Gly Pro Leu Ala Leu Val Asp Lys Leu Met Pro Val Ile 595 600 605

Met Ile Ile Met Arg Asp His Thr Tyr Ala Lys Cys Gln Asn Ala Leu 610 615 620

Gln Gln Val Val Ala Arg Gln Gly Arg Pro Val Val Ile Cys Asp Lys 625 630 635 640

Glu Asp Thr Glu Thr Ile Lys Asn Thr Lys Arg Thr Ile Lys Val Pro 645 650 655

His Ser Val Asp Cys Leu Gln Gly Ile Leu Ser Val Ile Pro Leu Gln 660 665 670

Leu Leu Ala Phe His Leu Ala Val Leu Arg Gly Tyr Asp Val Asp Phe 675 680 685

Pro Arg Asn Leu Ala Lys Ser Val Thr Val Glu 690 695

<210> 7

<211> 2064

<212> DNA

<213> Artificial sequence

<220>

<223> modified GFAT1 by an internal purification tag

<220>

<221> CDS

<222> (1)..(2064)

<223>

<220>

<221> misc_feature

<222> (170)..(170)

<223> t or c

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					acc Thr											96
					gct Ala											144
					gcc Ala											192
					gat Asp 70											240
_	_		_		gat Asp	-										288
					ccc Pro											336
					ttt Phe											384
					aag Lys											432
					gag Glu 150											480
					caa Gln											528
					gaa Glu											576
					gca Ala											624
					gaa Glu											672
ctc	tac	aga	aca	ggc	aaa	gac	aag	aaa	gga	agc	tgc	aat	ctc	tct	cgt	720

Leu 225	Tyr	Arg	Thr	Gly	Lys 230	Asp	Lys	Lys	Gly	Ser 235	Cys	Asn	Leu	Ser	Arg 240	
gtg Val	gac Asp	agc Ser	aca Thr	acc Thr 245	tgc Cys	ctt Leu	ttc Phe	ccg Pro	gtg Val 250	gaa Glu	gaa Glu	aaa Lys	gca Ala	gtg Val 255	gag Glu	768
tat Tyr	tac Tyr	ttt Phe	gct Ala 260	tct Ser	gat Asp	gca Ala	agt Ser	gct Ala 265	gtc [·] Val	ata Ile	gaa Glu	cac His	acc Thr 270	aat Asn	cgc Arg	816
gtc Val	atc Ile	ttt Phe 275	ctg Leu	gaa Glu	gat Asp	gat Asp	gat Asp 280	gtt Val	gca Ala	gca Ala	gta Val	gtg Val 285	gat Asp	gga Gly	cgt Arg	864
ctt Leu	tct Ser 290	atc Ile	cat His	cga Arg	att Ile	aaa Lys 295	cga Arg	act Thr	gca Ala	gga Gly	cat His 300	cac His	cat His	cac His	cat His	912
cac His 305	gat Asp	cac His	ccc Pro	gga Gly	cga Arg 310	gct Ala	gtg Val	caa Gln	aca Thr	ctc Leu 315	cag Gln	atg Met	gaa Glu	ctc Leu	cag Gln 320	960
cag Gln	atc Ile	atg Met	aag Lys	ggc Gly 325	aac Asn	ttc Phe	agt Ser	tca Ser	ttt Phe 330	atg Met	cag Gln	aag Lys	gaa Glu	ata Ile 335	ttt Phe	1008
gag Glu	cag Gln	cca Pro	gag Glu 340	tct Ser	gtc Val	gtg Val	aac Asn	aca Thr 345	atg Met	aga Arg	gga Gly	aga Arg	gtc Val 350	aac Asn	ttt Phe	1056
gat Asp	gac Asp	tat Tyr 355	act Thr	gtg Val	aat Asn	ttg Leu	ggt Gly 360	ggt Gly	ttg Leu	aag Lys	gat Asp	cac His 365	ata Ile	aag Lys	gag Glu	1104
atc Ile	cag Gln 370	aga Arg	tgc Cys	cgg Arg	cgt Arg	ttg Leu 375	att Ile	ctt Leu	att Ile	gct Ala	tgt Cys 380	gga Gly	aca Thr	agt Ser	tac Tyr	1152
cat His 385	Ala	ggt Gly	gta Val	gca Ala	aca Thr 390	cgt Arg	Gln	Val	Leu	gag Glu 395	gag Glu	ctg Leu	act Thr	gag Glu	ttg Leu 400	1200
cct Pro	gtg Val	atg Met	gtg Val	gaa Glu 405	cta Leu	gca Ala	agt Ser	gac Asp	ttc Phe 410	ctg Leu	gac Asp	aga Arg	aac Asn	aca Thr 415	cca Pro	1248
gtc Val	ttt Phe	cga Arg	gat Asp 420	gat Asp	gtt Val	tgc Cys	ttt Phe	ttc Phe 425	ctt Leu	agt Ser	caa Gln	tca Ser	ggt Gly 430	gag Glu	aca Thr	1296
gca Ala	gat Asp	act Thr 435	ttg Leu	atg Met	ggt Gly	ctt Leu	cgt Arg 440	tac Tyr	tgt Cys	aag Lys	gag Glu	aga Arg 445	gga Gly	gct Ala	tta Leu	1344
act Thr	gtg Val 450	ggg	atc Ile	aca Thr	aac Asn	aca Thr 455	gtt Val	ggc Gly	agt Ser	tcc Ser	ata Ile 460	tca Ser	cgg Arg	gag Glu	aca Thr	1392
gat Asp	tgt Cys	gga Gly	gtt Val	cat His	att Ile	aat Asn	gct Ala	ggt Gly	cct Pro	gag Glu	att Ile	ggt Gly	gtg Val	gcc Ala	agt Ser	1440

			•													
465					470			÷		475					480	
aca Thr	aag Lys	gct Ala	tat Tyr	acc Thr 485	agc Ser	cag Gln	ttt Phe	gta Val	tcc Ser 490	ctt Leu	gtg Val	atg Met	ttt Phe	gcc Ala 495	ctt Leu	1488
atg Met	atg Met	tgt Cys	gat Asp 500	gat Asp	cgg Arg	atc Ile	tcc Ser	atg Met 505	caà Gln	gaa Glu	aga Arg	cgc Arg	aaa Lys 510	gag Glu	atc Ile	1536
atg Met	ctt Leu	gga Gly 515	ttg Leu	aaa Lys	cgg Arg	ctg Leu	cct Pro 520	gat Asp	ttg Leu	att Ile	aàg Lys	gaa Glu 525	gta Val	ctg Leu	agc Ser	1584
atg Met	gat Asp 530	gac Asp	gaa Glu	att Ile	cag Gln	aaa Lys 535	cta Leu	gca Ala	aca Thr	gaa Glu	ctt Leu 540	tat Tyr	cat	cag Gln	aag Lys	1632
tca Ser 545	gtt Val	ctg Leu	ata Ile	atg Met	gga Gly 550	cga Arg	ggc Gly	tat Tyr	cat His	tat Tyr 555	gct Ala	act Thr	tgt Cys	ctt Leu	gaa Glu 560	1680
ggg Gly	gca Ala	ctg Leu	aaa Lys	atc Ile 565	aaa Lys	gaa Glu	att Ile	act Thr	tat Tyr 570	atg Met	cac His	tct Ser	gaa Glu	ggc Gly 575	atc Ile	1728
ctt Leu	gct Ala	ggt Gly	gaa Glu 580	ttg Leu	aaa Lys	cat His	ggc Gly	cct Pro 585	ctg Leu	gct Ala	ttg Leu	gtg Val	gat Asp 590	aaa Lys	ttg Leu	1776
atg Met	cct Pro	gtg Val 595	atc Ile	atg Met	atc Ile	atc Ile	atg Met 600	aga Arg	gat Asp	cac His	act Thr	tat Tyr 605	gcc Ala	aag Lys	tgt Cys	1824
cag Gln	aat Asn 610	gct Ala	ctt Leu	cag Gln	caa Gln	gtg Val 615	gtt Val	gct Ala	cgg Arg	cag Gln	ggg Gly 620	cgg Arg	cct Pro	gtg Val	gta Val	1872
att Ile 625	tgt Cys	gat Asp	aag Lys	gag Glu	gat Asp 630	act Thr	gag Glu	acc Thr	att Ile	aag Lys 635	aac Asn	aca Thr	aaa Lys	aga Arg	acg Thr 640	1920
atc Ile	aag Lys	gtg Val	ccc Pro	cac His 645	tca Ser	gtg Val	gac Asp	tgc Cys	ttg Leu 650	cag Gln	ggc Gly	att Ile	ctc Leu	agc Ser 655	gtg Val	1968
atc Ile	cct Pro	tta Leu	cag Gln 660	ttg Leu	ctg Leu	gct Ala	ttc Phe	cac His 665	ctt Leu	gct Ala	gtg Val	ctg Leu	aga Arg 670	ggc Gly	tat Tyr	2016
gat Asp	gtt Val	gat Asp 675	ttc Phe	cca Pro	cgg Arg	aat Asn	ctt Leu 680	gcc Ala	aaa Lys	tct Ser	gtg Val	act Thr 685	Val	gag Glu	tga	2064
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<213> Artificial sequence

<220>

<221> misc_feature

<222> (57)..(57)

<223> 'Xaa' in position 57 represents Thr or Ile

<220>

<223> modified GFAT1 by an internal purification tag

<400> 8

Met Cys Gly Ile Phe Ala Tyr Leu Asn Tyr His Val Pro Arg Thr Arg 1 5 10 15

Arg Glu Ile Leu Glu Thr Leu Ile Lys Gly Leu Gln Arg Leu Glu Tyr 20 25 30

Arg Gly Tyr Asp Ser Ala Gly Val Gly Phe Asp Gly Gly Asn Asp Lys
35 40 45

Asp Trp Glu Ala Asn Ala Cys Lys Xaa Gln Leu Île Lys Lys Gly 50 60

Lys Val Lys Ala Leu Asp Glu Glu Val His Lys Gln Gln Asp Met Asp 65 70 75 . 80

Leu Asp Ile Glu Phe Asp Val His Leu Gly Ile Ala His Thr Arg Trp 85 90 95

Ala Thr His Gly Glu Pro Ser Pro Val Asn Ser His Pro Gln Arg Ser 100 105 110

Asp Lys Asn Asn Glu Phe Ile Val Ile His Asn Gly Ile Ile Thr Asn 115 120 125

Tyr Lys Asp Leu Lys Lys Phe Leu Glu Ser Lys Gly Tyr Asp Phe Glu 130 135 140

Ser Glu Thr Asp Thr Glu Thr Ile Ala Lys Leu Val Lys Tyr Met Tyr 145 150 155 160

Asp Asn Arg Glu Ser Gln Asp Thr Ser Phe Thr Thr Leu Val Glu Arg 165 170 175

Val Ile Gln Gln Leu Glu Gly Ala Phe Ala Leu Val Phe Lys Ser Val 180 185 190

His Phe Pro Gly Gln Ala Val Gly Thr Arg Arg Gly Ser Pro Leu Leu 195 200 205

Ile Gly Val Arg Ser Glu His Lys Leu Ser Thr Asp His Ile Pro Ile 210 215 220

Leu Tyr Arg Thr Gly Lys Asp Lys Gly Ser Cys Asn Leu Ser Arg 225 230 . 235 240

Val Asp Ser Thr Thr Cys Leu Phe Pro Val Glu Glu Lys Ala Val Glu 245 250 255

Tyr Tyr Phe Ala Ser Asp Ala Ser Ala Val Ile Glu His Thr Asn Arg 260 265 270

Val Ile Phe Leu Glu Asp Asp Asp Val Ala Ala Val Val Asp Gly Arg

275 280 285

Leu Ser Ile His Arg Ile Lys Arg Thr Ala Gly His His His His His His Asp His Pro Gly Arg Ala Val Gln Thr Leu Gln Met Glu Leu Gln 315 Gln Ile Met Lys Gly Asn Phe Ser Ser Phe Met Gln Lys Glu Ile Phe 325 330 Glu Gln Pro Glu Ser Val Val Asn Thr Met Arg Gly Arg Val Asn Phe 340 345 Asp Asp Tyr Thr Val Asn Leu Gly Gly Leu Lys Asp His Ile Lys Glu 360 Ile Gln Arg Cys Arg Arg Leu Ile Leu Ile Ala Cys Gly Thr Ser Tyr 375 His Ala Gly Val Ala Thr Arg Gln Val Leu Glu Glu Leu Thr Glu Leu 390 395 Pro Val Met Val Glu Leu Ala Ser Asp Phe Leu Asp Arg Asn Thr Pro Val Phe Arg Asp Asp Val Cys Phe Phe Leu Ser Gln Ser Gly Glu Thr 425 Ala Asp Thr Leu Met Gly Leu Arg Tyr Cys Lys Glu Arg Gly Ala Leu 435 Thr Val Gly Ile Thr Asn Thr Val Gly Ser Ser Ile Ser Arg Glu Thr 455 Asp Cys Gly Val His Ile Asn Ala Gly Pro Glu Ile Gly Val Ala Ser 470 Thr Lys Ala Tyr Thr Ser Gln Phe Val Ser Leu Val Met Phe Ala Leu 485 490 Met Met Cys Asp Asp Arg Ile Ser Met Gln Glu Arg Arg Lys Glu Ile 500 Met Leu Gly Leu Lys Arg Leu Pro Asp Leu Ile Lys Glu Val Leu Ser Met Asp Asp Glu Ile Gln Lys Leu Ala Thr Glu Leu Tyr His Gln Lys 530 Ser Val Leu Ile Met Gly Arg Gly Tyr His Tyr Ala Thr Cys Leu Glu Gly Ala Leu Lys Ile Lys Glu Ile Thr Tyr Met His Ser Glu Gly Ile Leu Ala Gly Glu Leu Lys His Gly Pro Leu Ala Leu Val Asp Lys Leu Met Pro Val Ile Met Ile Ile Met Arg Asp His Thr Tyr Ala Lys Cys

595 600 605

Gln Asn Ala Leu Gln Gln Val Val Ala Arg Gln Gly Arg Pro Val Val 615 610 Ile Cys Asp Lys Glu Asp Thr Glu Thr Ile Lys Asn Thr Lys Arg Thr 630 Ile Lys Val Pro His Ser Val Asp Cys Leu Gln Gly Ile Leu Ser Val 645 650 Ile Pro Leu Gln Leu Leu Ala Phe His Leu Ala Val Leu Arg Gly Tyr 665 Asp Val Asp Phe Pro Arg Asn Leu Ala Lys Ser Val Thr Val Glu <210> 9 <211> 2067 <212> DNA <213> Artificial sequence <220> <223> modified GFAT2 by an internal purification tag <220> <221> CDS (1)..(2067)<222> <223> <400> 9 48 atg tgc gga atc ttt gcc tac atg aac tac aga gtc ccc cgg acg agg Met Cys Gly Ile Phe Ala Tyr Met Asn Tyr Arg Val Pro Arg Thr Arg 96 aag gag atc ttc gaa acc ctc atc aag ggc ctg cag cgg ctg gag tac Lys Glu Ile Phe Glu Thr Leu Ile Lys Gly Leu Gln Arg Leu Glu Tyr aga ggc tac gac tcg gca ggt gtg gcg atc gat ggg aat aat cac gaa 144 Arg Gly Tyr Asp Ser Ala Gly Val Ala Ile Asp Gly Asn Asn His Glu 35 192 gtc aaa gaa aga cac att cag ctg gtc aag aaa agg ggg aaa gtc aag Val Lys Glu Arg His Ile Gln Leu Val Lys Lys Arg Gly Lys Val Lys 50 55 gct ctc gat gaa gaa ctt tac aaa caa gac agc atg gac tta aaa gtg 240 Ala Leu Asp Glu Glu Leu Tyr Lys Gln Asp Ser Met Asp Leu Lys Val 65 gag ttt gag aca cac ttc ggc att gcc cac acg cgc tgg gcc acc cac Glu Phe Glu Thr His Phe Gly Ile Ala His Thr Arg Trp Ala Thr His 85 ggg gtc ccc agt gct gtc aac agc cac cct cag cgc tca gac aaa ggc 336 Gly Val Pro Ser Ala Val Asn Ser His Pro Gln Arg Ser Asp Lys Gly 100 105

			Val					Gly					tac Tyr			384	
		Lys											tca Ser			432	
	Thr				-	Lys	_						gac Asp		_	480	
													gtc Val			528	
													cac His 190			576	
													atc Ile			624	
													tta Leu			672	
													cgg Arg			720	
agg Arg	ctg Leu	gac Asp	agc Ser	tcc Ser 245	gcc Ala	tgc Cys	ctg Leu	cat His	gct Ala 250	gtg Val	ggc Gly	gac Asp	aag Lys	gcc Ala 255	gtg Val	768	
gaa Glu	ttc Phe	ttc Phe	ttt Phe 260	gct Ala	tct Ser	gat Asp	gca Ala	agc Ser 265	gct Ala	atc Ile	ata Ile	gag Glu	cac His 270	acc Thr	aac Asn	816	
_					_								gct Ala	-		864	
aaa Lys	ctc Leu 290	tcc Ser	att Ile	cac His	cgg Arg	gtc Val 295	aag Lys	cgc Arg	tcg Ser	gcc Ala	agt Ser 300	cat His	cac His	cat His	cac His	912	
cat His 305	cac His	gat Asp	gac Asp	cca Pro	tct Ser 310	cga Arg	gcc Ala	atc Ile	cag Gln	acc Thr 315	ttg Leu	cag Gln	atg Met	gaa Glu	ctg Leu 320	960	
cag Gln	caa Gln	atc Ile	atg Met	aaa Lys 325	ggt Gly	aac Asn	ttc Phe	agt Ser	gcg Ala 330	ttt Phe	atg Met	cag Gln	aag Lys	gag Glu 335	atc Ile	1008	
ttc Phe	gaa Glu	cag Gln	cca Pro 340	gaa Glu	tca Ser	gtt Val	ttc Phe	aat Asn 345	act Thr	atg Met	aga Arg	ggt Gly	cgg Arg 350	gtg Val	aat Asn	1056	
ttt	gaa	acc	aac	aca	gtg	ctc	ctg	ggt	ggc	ttg	aag	gac	cac	ttg	aag	1104	

Phe	Glu	Thr 355	Asn	Thr	Val	Leu	Leu 360	Gly	Gly	Leu	Lys	Asp 365	His	Leu	Lys		
						cgg Arg 375										•	1152
	His					acg Thr											1200
			_	_	_	ctt Leu	_	_	_		_	_					1248
				-	-	gtt Val	_				_	_					1296
						gcg Ala					Lys						1344
						aac Asn 455											1392
						atc Ile											1440
						agt Ser											1488
						cga Arg											1536
		Arg	Gly	Leu	Arg	tct Ser	Leu	Pro	Glu	Leu	Ile	Lys	Glu				1584
						cac His 535											1632
						gjy ggg											1680
						aaa Lys											1728
						aag Lys											1776
						gtc Val											1824

595 600 605

tgc cag aac o Cys Gln Asn 7 610			Val			Arg G						1872
ata ctg tgc : Ile Leu Cys : 625					Ser S							1920
aca atc gag of Thr Ile Glu				Asp								1968
gtg att ccg (Val Ile Pro I												2016
tat gac gtt o Tyr Asp Val 2 675												2064
tga												2067
<210> 10 <211> 688 <212> PRT <213> Artif:	icial se	equence										
<220> <223> modif:	ied GFAT	T2 by an	inte	rnal	puri	ifica	atio	n ta	ıg			
		_		Asn						Thr 15	Arg	
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Asp Thr Glu Thr Ile Ala Lys Leu Ile Lys Tyr Val Phe Asp Asn Arg 155 Glu Thr Glu Asp Ile Thr Phe Ser Thr Leu Val Glu Arg Val Ile Gln 165 Gln Leu Glu Gly Ala Phe Ala Leu Val Phe Lys Ser Val His Tyr Pro 185 180 Gly Glu Ala Val Ala Thr Arg Arg Gly Ser Pro Leu Leu Ile Gly Val 200 Arg Ser Lys Tyr Lys Leu Ser Thr Glu Gln Ile Pro Ile Leu Tyr Arg 215 Thr Cys Thr Leu Glu Asn Val Lys Asn Ile Cys Lys Thr Arg Met Lys 230 235 Arg Leu Asp Ser Ser Ala Cys Leu His Ala Val Gly Asp Lys Ala Val Glu Phe Phe Ala Ser Asp Ala Ser Ala Ile Ile Glu His Thr Asn Arg Val Ile Phe Leu Glu Asp Asp Ile Ala Ala Val Ala Asp Gly 280 Lys Leu Ser Ile His Arg Val Lys Arg Ser Ala Ser His His His His 295 His His Asp Asp Pro Ser Arg Ala Ile Gln Thr Leu Gln Met Glu Leu 310 315 Gln Gln Ile Met Lys Gly Asn Phe Ser Ala Phe Met Gln Lys Glu Ile 325 Phe Glu Gln Pro Glu Ser Val Phe Asn Thr Met Arg Gly Arg Val Asn 345 Phe Glu Thr Asn Thr Val Leu Leu Gly Gly Leu Lys Asp His Leu Lys 355 Glu Ile Arg Arg Cys Arg Arg Leu Ile Val Ile Gly Cys Gly Thr Ser 375 Tyr His Ala Ala Val Ala Thr Arg Gln Val Leu Glu Glu Leu Thr Glu Leu Pro Val Met Val Glu Leu Ala Ser Asp Phe Leu Asp Arg Asn Thr Pro Val Phe Arg Asp Asp Val Cys Phe Phe Ile Ser Gln Ser Gly Glu Thr Ala Asp Thr Leu Leu Ala Leu Arg Tyr Cys Lys Asp Arg Gly Ala Leu Thr Val Gly Val Thr Asn Thr Val Gly Ser Ser Ile Ser Arg Glu 455

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Thr Asp Cys Gly Val His Ile Asn Ala Gly Pro Glu Val Gly Val Ala
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Ser Thr Lys Ala Tyr Thr Ser Gln Phe Ile Ser Leu Val Met Phe Gly
                                    490
                485
Leu Met Met Ser Glu Asp Arg Ile Ser Leu Gln Asn Arg Arg Gln Glu
                                505
Ile Ile Arg Gly Leu Arg Ser Leu Pro Glu Leu Ile Lys Glu Val Leu
                            520
Ser Leu Glu Glu Lys Ile His Asp Leu Ala Leu Glu Leu Tyr Thr Gln
                        535
Arg Ser Leu Leu Val Met Gly Arg Gly Tyr Asn Tyr Ala Thr Cys Leu
                                        555
Glu Gly Ala Leu Lys Ile Lys Glu Ile Thr Tyr Met His Ser Glu Gly
                                    570
Ile Leu Ala Gly Glu Leu Lys His Gly Pro Leu Ala Leu Ile Asp Lys
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Gln Met Pro Val Ile Met Val Ile Met Lys Asp Pro Cys Phe Ala Lys
       595
Cys Gln Asn Ala Leu Gln Gln Val Thr Ala Arg Gln Gly Arg Pro Ile
                       615
Ile Leu Cys Ser Lys Asp Asp Thr Glu Ser Ser Lys Phe Ala Tyr Lys
Thr Ile Glu Leu Pro His Thr Val Asp Cys Leu Gln Gly Ile Leu Ser
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Tyr Asp Val Asp Phe Pro Arg Asn Leu Ala Lys Ser Val Thr Val Glu
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			ctt cag aga ctg Leu Gln Arg Leu 30		
aga gga tat gat Arg Gly Tyr Asp 35	tct gct ggt Ser Ala Gly	gtg gga ttt Val Gly Phe 40	gat gga ggc aat Asp Gly Gly Asn 45	gat aaa 144 Asp Lys	
gat tgg gaa gcc Asp Trp Glu Ala 50	aat gcc tgc Asn Ala Cys 55	aaa anc cag Lys Xaa Gln	ctt att aag aag Leu Ile Lys Lys 60	aaa gga 192 Lys Gly	
aaa gtt aag gca Lys Val Lys Ala 65	ctg gat gaa Leu Asp Glu 70	gaa gtt cac Glu Val His	aag caa caa gat Lys Gln Gln Asp 75	atg gat 240 Met Asp 80	
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gca aca cat gga Ala Thr His Gly 100	Glu Pro Ser	cct gtc aat Pro Val Asn 105	agc cac ccc cag Ser His Pro Gln 110	cgc tct 336 Arg Ser	
gat aaa aat aat Asp Lys Asn Asr 115	gaa ttt ato Glu Phe Ile	gtt att cac Val Ile His 120	aat gga atc atc Asn Gly Ile Ile 125	acc aac 384 Thr Asn	
tac aaa gac tto Tyr Lys Asp Lei 130	g aaa aag ttt Lys Lys Phe 135	Leu Glu Ser	aaa ggc tat gac Lys Gly Tyr Asp 140	ttc gaa 432 Phe Glu	
			ctc gtt aag tat Leu Val Lys Tyr 155		
			act acc ttg gtg Thr Thr Leu Val		
gtt atc caa caa Val Ile Gln Glr 180	Leu Glu Gl	gct ttt gca Ala Phe Ala 185	ctt gtg ttt aaa Leu Val Phe Lys 190	agt gtt 576 Ser Val	
			cga ggt agc cct Arg Gly Ser Pro 205		
		Lys Leu Ser	act gat cac att Thr Asp His Ile 220		
ctc tac aga aca	ı get agg act	cag att gga	tca aaa ttc aca	cgg tgg 720	

Leu 225	-	Arg	Thr	Ala	Arg 230	Thr	Gln	Ile	Gly	Ser 235	Lys	Phe	Thr	Arg	Trp 240	
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	_		gac Asp 260	_			_			_		_	-		_	816
			tac Tyr													864
			atc Ile													912
	_		tct Ser						_		_			His		960
			gat Asp													1008
			atc Ile 340													1056
			cag Gln													1104
			gac Asp													1152
	Glu		cag Gln	Arg		Arg		Leu	Ile		Ile					1200
			gct Ala													1248
			gtg Val 420													1296
			ttt Phe													1344
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			gtg Val													1440

465					470					475					480	
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						acc Thr										1536
						gat Asp										1584
						aaa Lys 535										1632
	Ser					att Ile										1680
						atg Met										1728
						atc Ile										1776
						ttg Leu										1824
						atg Met 615										1872
aag Lys 625	tgt Cys	cag Gln	aat Asn	gct Ala	ctt Leu 630	cag Gln	caa Gln	gtg Val	gtt Val	Ala	Arg	cag Gln	Gly	Arg	cct Pro 640	1920
						gag Glu										1968
aga Arg	acg Thr	atc Ile	aag Lys 660	gtg Val	ccc Pro	cac His	tca Ser	gtg Val 665	gac Asp	tgc Cys	ttg Leu	cag Gln	ggc Gly 670	att Ile	ctc Leu	2016
`agc Ser	gtg Val	atc Ile 675	cct Pro	tta Leu	cag Gln	ttg Leu	ctg Leu 680	gct Ala	ttc Phe	cac His	ctt Leu	gct Ala 685	gtg Val	ctg Leu	aga Arg	2064
Gly	tat Tyr 690	gat Asp	gtt Val	gat Asp	ttc Phe	cca Pro 695	cgg Arg	aat Asn	ctt Leu	gcc Ala	aaa Lys 700	tct Ser	gtg Val	act Thr	gta Val	2112
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705

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Gly Ser Gln Gly Glu Arg Gly Lys Asp Lys Lys Gly Ser Cys Asn Leu 245 Ser Arg Val Asp Ser Thr Thr Cys Leu Phe Pro Val Glu Glu Lys Ala 265 Val Glu Tyr Tyr Phe Ala Ser Asp Ala Ser Ala Val Ile Glu His Thr 280 Asn Arg Val Ile Phe Leu Glu Asp Asp Val Ala Ala Val Val Asp 295 Gly Arg Leu Ser Ile His Arg Ile Lys Arg Thr Ala Gly His His His 315 His His His Asp His Pro Gly Arg Ala Val Gln Thr Leu Gln Met Glu 325 Leu Gln Gln Ile Met Lys Gly Asn Phe Ser Ser Phe Met Gln Lys Glu 345 Ile Phe Glu Gln Pro Glu Ser Val Val Asn Thr Met Arg Gly Arg Val Asn Phe Asp Asp Tyr Thr Val Asn Leu Gly Gly Leu Lys Asp His Ile 375 Lys Glu Ile Gln Arg Cys Arg Arg Leu Ile Leu Ile Ala Cys Gly Thr Ser Tyr His Ala Gly Val Ala Thr Arg Gln Val Leu Glu Glu Leu Thr 410 Glu Leu Pro Val Met Val Glu Leu Ala Ser Asp Phe Leu Asp Arg Asn Thr Pro Val Phe Arg Asp Asp Val Cys Phe Phe Leu Ser Gln Ser Gly Glu Thr Ala Asp Thr Leu Met Gly Leu Arg Tyr Cys Lys Glu Arg Gly Ala Leu Thr Val Gly Ile Thr Asn Thr Val Gly Ser Ser Ile Ser Arg Glu Thr Asp Cys Gly Val His Ile Asn Ala Gly Pro Glu Ile Gly Val 485 Ala Ser Thr Lys Ala Tyr Thr Ser Gln Phe Val Ser Leu Val Met Phe 505 Ala Leu Met Met Cys Asp Asp Arg Ile Ser Met Gln Glu Arg Arg Lys 520 515 Glu Ile Met Leu Gly Leu Lys Arg Leu Pro Asp Leu Ile Lys Glu Val 535 Leu Ser Met Asp Asp Glu Ile Gln Lys Leu Ala Thr Glu Leu Tyr His

550

Gln Lys Ser Val Leu Ile Met Gly Arg Gly Tyr His Tyr Ala Thr Cys 575

Leu Glu Gly Ala Leu Lys Ile Lys Glu Ile Thr Tyr Met His Ser Glu

Gly Ile Leu Ala Gly Glu Leu Lys His Gly Pro Leu Ala Leu Val Asp 595 600 605

Lys Leu Met Pro Val Ile Met Ile Ile Met Arg Asp His Thr Tyr Ala 610 615 620

Lys Cys Gln Asn Ala Leu Gln Gln Val Val Ala Arg Gln Gly Arg Pro 625 630 635 640

Val Val Ile Cys Asp Lys Glu Asp Thr Glu Thr Ile Lys Asn Thr Lys 645 650 655

Arg Thr Ile Lys Val Pro His Ser Val Asp Cys Leu Gln Gly Ile Leu 660 665 670

Ser Val Ile Pro Leu Gln Leu Leu Ala Phe His Leu Ala Val Leu Arg 675 680 685

Gly Tyr Asp Val Asp Phe Pro Arg Asn Leu Ala Lys Ser Val Thr Val 690 695 700

Glu 705

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<211> 608

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<213> Escherichia coli

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Leu Glu Gly Leu Arg Arg Leu Glu Tyr Arg Gly Tyr Asp Ser Ala Gly 20 25 30

Leu Ala Val Val Asp Ala Glu Gly His Met Thr Arg Leu Arg Arg Leu 35 40 45

Gly Lys Val Gln Met Leu Ala Gln Ala Ala Glu Glu His Pro Leu His 50 55 60

Gly Gly Thr Gly Ile Ala His Thr Arg Trp Ala Thr His Gly Glu Pro 75 80

Ser Glu Val Asn Ala His Pro His Val Ser Glu His Ile Val Val 85 90 95

His Asn Gly Ile Ile Glu Asn His Glu Pro Leu Arg Glu Glu Léu Lys 100 105 110

Ala Arg Gly Tyr Thr Phe Val Ser Glu Thr Asp Thr Glu Val Ile Ala 115 120 125 His Leu Val Asn Trp Glu Leu Lys Gln Gly Gly Thr Leu Arg Glu Ala 135 Val Leu Arg Ala Ile Pro Gln Leu Arg Gly Ala Tyr Gly Thr Val Ile 150 Met Asp Ser Arg His Pro Asp Thr Leu Leu Ala Ala Arg Ser Gly Ser 170 165 Pro Leu Val Ile Gly Leu Gly Met Gly Glu Asn Phe Ile Ala Ser Asp 185 Gln Leu Ala Leu Leu Pro Val Thr Arg Arg Phe Ile Phe Leu Glu Glu 200 Gly Asp Ile Ala Glu Ile Thr Arg Arg Ser Val Asn Ile Phe Asp Lys 215 Thr Gly Ala Glu Val Lys Arg Gln Asp Ile Glu Ser Asn Leu Gln Tyr 235 Asp Ala Gly Asp Lys Gly Ile Tyr Arg His Tyr Met Gln Lys Glu Ile 250 Tyr Glu Gln Pro Asn Ala Ile Lys Asn Thr Leu Thr Gly Arg Ile Ser His Gly Gln Val Asp Leu Ser Glu Leu Gly Pro Asn Ala Asp Glu Leu 280 Leu Ser Lys Val Glu His Ile Gln Ile Leu Ala Cys Gly Thr Ser Tyr 295 Asn Ser Gly Met Val Ser Arg Tyr Trp Phe Glu Ser Leu Ala Gly Ile 315 Pro Cys Asp Val Glu Ile Ala Ser Glu Phe Arg Tyr Arg Lys Ser Ala Val Arg Arg Asn Ser Leu Met Ile Thr Leu Ser Gln Ser Gly Glu Thr Ala Asp Thr Leu Ala Gly Leu Arg Leu Ser Lys Glu Leu Gly Tyr Leu 355 Gly Ser Leu Ala Ile Cys Asn Val Pro Gly Ser Ser Leu Val Arg Glu Ser Asp Leu Ala Leu Met Thr Asn Ala Gly Thr Glu Ile Gly Val Ala 385 390 Ser Thr Lys Ala Phe Thr Thr Gln Leu Thr Val Leu Leu Met Leu Val 405 Ala Lys Leu Ser Arg Leu Lys Gly Leu Asp Ala Ser Ile Glu His Asp Ile Val His Gly Leu Gln Ala Leu Pro Ser Arg Ile Glu Gln Met Leu 435 Ser Gln Asp Lys Arg Ile Glu Ala Leu Ala Glu Asp Phe Ser Asp Lys

455 460 450 His His Ala Leu Phe Leu Gly Arg Gly Asp Gln Tyr Pro Ile Ala Leu 470 Glu Gly Ala Leu Lys Leu Lys Glu Ile Ser Tyr Ile His Ala Glu Ala 490 485 Tyr Ala Ala Gly Glu Leu Lys His Gly Pro Leu Ala Leu Ile Asp Ala 505 Asp Met Pro Val Ile Val Val Ala Pro Asn Asn Glu Leu Leu Glu Lys 520 Leu Lys Ser Asn Ile Glu Glu Val Arg Ala Arg Gly Gly Gln Leu Tyr 535 Val Phe Ala Asp Gln Asp Ala Gly Phe Val Ser Ser Asp Asn Met His Ile Ile Glu Met Pro His Val Glu Glu Val Ile Ala Pro Ile Phe Tyr 570 Thr Val Pro Leu Gln Leu Leu Ala Tyr His Val Ala Leu Ile Lys Gly 580 Thr Asp Val Asp Gln Pro Arg Asn Leu Ala Lys Ser Val Thr Val Glu 600 <210> 14 <211> 72 <212> DNA <213> Artificial sequence <220> <223> Primer tggacgtctt tctatccatc gaattaaacg aactgcagga catcaccatc accatcacga 60 72 tcaccccgga cg <210> 15 <211> 44 <212> DNA <213> Artificial sequence <220> <223> Primer <400> 15

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His His His His His
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